1550-Pos Board B501
Which Free Energy Methods can Predict Transport by Proton-Dependent Oligopeptide Symporters?
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Peptide transporters use a proton electrochemical gradient to move di- and tri-peptides across the membrane. Despite having crystal stuctures from bacteria, little is known on how these transporters achieve a broad substrate specificity. In this study, we modelled the binding of peptides and drugs on peptide transporters based on recently published crystal structures [1] and used a range of free energy methods to predict transport. The binding free energies calculated were compared to IC50 values from in vitro transport assay. We found that while docking scores produced inaccurate results, a reasonable correlation can be achieved by end-point methods such as molecular mechanics Poisson Boltzmann surface area (MMPBSA). Theoretically-rigorous methods such as thermodynamic integration (TI) can subsequently be employed to fine-tune the predictions amongst substrates with similar transport properties. Our calculations reproduced this transporter's preference for hydrophobic ligands (due to a hydrophobic pocket formed by Tyr68 and Trp296), while charged substrates showed lower affinity as they can disrupt existing salt bridges in the protein. Overall, our study reveals the potential use of free energy methods to predict the transport of small molecules by a symporter protein and help understand its substrate selectivity.
[1] Lyons, J. ; Parker, J. ; Solcan, N. ; Brinth, A. ; Li, D. ; Shah, S. ; Caffrey, M. ; Newstead, S. ; EMBO Rep., 15 (8), 886-893, 2014

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Mechanistic Details of Drug Translocation in MexAB-OprM Efflux Pump
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Antibiotic efflux is one of the most important mechanisms of bacterial multi-drug resistance. Antibiotics are pumped out of the bacterial cell by tripartite efflux pumps containing three protein components (outer-membrane, periplasmic, and inner-membrane proteins). Together they are able to actively extrude both noxious and hazardless compounds from the internal bacterial compartments to the extracellular environment.
Despite the efforts to characterize both the mechanism and dynamics of the tripartite pump complex, there are still several important answers that remain elusive. Here, we have used multi-scale molecular dynamics to get insights into the mechanism of drug translocation across the whole protein machinery. We have considered the MexAB-OprM pump complex of Pseudomonas aeruginosa in a lipid model membrane that contains many components of a gram-negative bacterium. Our results suggest that the drug translocation depends on the size of the molecule. While drugs with varying sizes enter through a vestibule accessible from periplasm, only smaller drugs can enter the pump through a channel from the cytoplasmic leaflet of the inner-membrane component. Overall, drug binding leads to subtle conformational changes that are transmitted along the components in the periplasmic region. The influence of such drug induced conformational changes on the periplasmic and outer-membrane components and the coupling to the proton motive force are being probed. Subsequent extrusion of the drug through the outer membrane component is a passive process and concentration dependent. The free energy landscape of the drug translocation through this pump is evaluated as well.

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Proton-Driven Molecular Ratchet Iteratively Activated by Microfluidics
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Anthrax toxin (AT), the key virulence factor secreted by Bacillus anthracis, injects lethal enzymes into cells using pH and voltage transmembrane gradients. Anthrax toxin is comprised of three proteins: protective antigen (PA, 83 kDa), the pore-forming protein which delivers the other two toxic enzymes - edema factor (EF, 89 kDa) and lethal factor (LF, 90 kDa) - into the cell cytosol. The mechanism of AT translocation is dependent on a pH gradient, but exactly how this works is not yet clear. Here, we use a droplet-interface bilayer(DIB)-based fluid exchange device that allows reagents to be introduced to the model membrane in rapid sequence. We are able to switch proton gradient across the membrane on and off and reload AT proteins repeatedly. To support the putative Brownian-ratchet mechanism of translocation, the AT translocation was induced using a gradient and subsequently paused. By cycling the gradient on and off, the translocation can be started and stopped repeatedly as shown in our electrical recordings. This method may be of utility in the study of other AB toxins that are believed to operate using a proton gradient for energy.

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Physical Picture for Functionally Rotating Mechanism of the Multidrug Efflux Transporter AcrB
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The multidrug efflux pump of *Escherichia coli* is the tripartite complex consisting of the polytopic inner membrane protein AcrB, periplasmic adaptor protein AcrA, and outer membrane channel TolC. AcrB is in charge of the principal part of pumping drugs out of the cell from the inner membrane or periplasm through the TolC channel. AcrB comprises three porotomers with different conformations which are in access (A), binding (B), and extrusion (E) states along the drug transport cycle, respectively. A three-step ''functionally rotating'' picture has then been proposed for the transport of drugs: Each protomer exhibits a sequential structural change represented as (A, B, E)→(B, E, A)→(E, A, B)→(A, B, E) by utilizing the proton-motive force. Up to now, MD simulations have been performed extensively to elucidate the rotating mechanism. However, they are focused on particular aspects or elementary processes in the functional rotation cycle. The mechanism of the functional rotation and energetics of the whole cycle remain unresolved. Here we investigate the packing structure of AcrB in terms of the entropic effect originating from the translational displacement of water molecules or hydrocarbon groups constituting nonpolar chains of lipid molecules. The theoretical method, which consists of the integral equation theories for simple and molecular fluids combined with the morphometric approach, allows us to analyze solvation properties of the trimer as well as each protomer by accounting for the polyatomic structures in atomic details. We find that the packing in AcrB is highly asymmetric and the solvent-entropy effect is crucially important. We construct a physical picture for the mechanism of conformational rotation elucidating how each protomer achieves such a drastic conformational change using only a small free energy ~$8k_BT$.

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Molecular Physiology of Uncoupling Proteins in the Central Nervous System: Self-Association and Proton Transport
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Uncoupling proteins (UCPs), located in the mitochondrial inner membrane, facilitate the transmembrane proton flux and, consequently, reduce the membrane potential and ATP production. Found in the central nervous system (CNS), based on several studies on animal models and clinical investigations, three human UCP homologs (UCP2, UCP4, and UCP5) could have crucial physiological functions. Despite these studies, the detailed structural features and molecular physiology landscape of these proteins remain relatively unexplored. Recently, we reported a novel expression system for obtaining functionally folded UCP1 in bacterial membranes (Hoang et al., 2013). In the current study, employing similar expression and reconstitution methods, we will report our new findings for the three human neuronal UCP homologs. The reconstituted CNS proteins display high helical contents and transport protons in the presence of several physiologically-relevant fatty acid activators. In addition, experimental results from CD, fluorescence spectroscopy, mass spectrometry and semi-native electrophoresis suggest self-association of these proteins in the membranes. While sharing comparable secondary structures in the liposomes, neuronal UCPs differ in their proton transport rates (and possibly mechanism) in the presence of different fatty acid activators. The protein-fatty acids interaction is further investigated using near-UV CD spectroscopy. The differences in fatty-acid activated UCP-mediated proton transport could serve as an essential clue in understanding and differentiating the physiological roles of UCP homologs in the CNS.
Hoang T, Smith MD, Jelokhani-Niaraki M (2013) Expression, folding, and proton transport activity of human uncoupling protein-1 (UCP1) in lipid membranes: evidence for associated functional forms. J. Biol. Chem. 288, 36244-36258